Neural crest progenitors and stem cells: From early development to adulthood

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Highlights

1. Upon isolation, many NC-derived cells (referred to as NCSCs) display stem cell features
2. Still debated is to what extent NCSCs exhibit self-renewing and multipotency in vivo
3. NCSC fates are regulated in a region- and stage-specific manner
4. NCSC-like progenitors persist in multiple adult tissues including skin and bone marrow
5. Adult NCSCs might be involved in tissue homeostasis and regeneration

Neural crest progenitors and stem cells: from early development to adulthood

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Abbreviations: NC, neural crest; NCC, neural crest cell; NCSC, neural crest stem cell; PNS, peripheral nervous system; DRG, dorsal root ganglia; Shh, Sonic Hedgehog; EDN3, endothelin-3; EMT, epithelial-mesenchymal transition; SKP, skin-derived precursor; CB, carotid body.

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Abstract

In the vertebrate embryo, the neural crest forms transiently in the dorsal neural primordium to yield migratory cells that will invade nearly all tissues and later, will differentiate into bones and cartilages, neurons and glia, endocrine cells, vascular smooth muscle cells and melanocytes. Due to the amazingly diversified array of cell types it produces, the neural crest is an attractive model system in the stem cell field. We present here in vivo and in vitro studies of single cell fate, which led to discovery and characterization of stem cells in the neural crest of avian and mammalian embryos. Some of the key issues in neural crest cell diversification are discussed, such as the time of segregation of mesenchymal vs. neural/melanocytic lineages, and the origin and close relationships between the glial and melanocytic lineages. An overview is also provided of the diverse types of neural crest-like stem cells and progenitors, recently identified in a growing number of adult tissues in animals and humans. Current and future work, in which in vivo lineage studies and the use of injury models will complement the in vitro culture analysis, should help unraveling the properties and function of neural crest-derived progenitors in development and disease.

1. The NCCs, a multi-fated embryonic cell population characteristic of vertebrates

The NCCs emerge in neurulating vertebrate embryo in the neural plate border region, between the presumptive neural epithelium and epidermis. The induction and specification of NC depend largely upon a set of signaling molecules and transcription factors whose actions are strikingly coordinated in time and space during gastrulation and neurulation stages (for references, see Milet and Monsoro-Burq, 2012, this issue). Current knowledge of the regulatory gene network involved in the induction and formation of the NC is reviewed elsewhere (Sauka-Spengler and Bronner-Fraser, 2008; Sauka-Spengler, 2012 this volume). Briefly, the neural border is first specified by the interplay of BMP, Wnt and FGF signalings originating in non-neural ectoderm and paraxial mesoderm. In turn, neural border genes up-regulate another set of transcription factor genes involved in NC specification, which control later cellular events like delamination, cell proliferation, migration and differentiation (for reviews, Kuriyama and Mayor, 2008; Nikitina et al., 2009).

Soon after individualization in the dorsal-most part of the closing neural tube, the NCCs undergo epithelial-to-mesenchymal transition (EMT), migrate along several stereotyped routes within embryonic tissues and finally settle and differentiate in various elected locations (Le Douarin and Kalcheim, 1999; Morales et al., 2005; Dupin et al., 2006 for reviews). A number of studies highlighted the migration behavior and motile properties of the NCC population and its individual cells and began to uncover the genetic determinants of the EMT in NCCs, which represents a model system of the dissemination and metastasis by tumor cells (reviewed by Théveneau and Mayor, 2012, this issue).

The invasive properties and lineage repertoire of the cells derived from the NC are quite impressive since they populate virtually all the tissues and generate a wide diversity of cell types, of the vertebrate body. The NC is at the origin of pigment cells, the melanocytes present in the vertebrate skin and the inner ear of mammals, at the exception of the retinal

pigment cells. The NC yields most of the peripheral nervous system (PNS) (neurons of the sensory, sympathetic, parasympathetic and enteric ganglia as well as ganglionic satellite glial cells and Schwann cells lining peripheral nerves) (Le Douarin and Kalcheim, 1999). The olfactory ensheathing cells of the olfactory nerve also derive from NCCs (Barraud et al., 2010). In addition, several types of neuro-glandular cells originate from the NC (for references, Adams and Bronner-Fraser, 2009). Besides neural and melanocytic cell types, the NC is the source of a variety of mesenchymal cells termed mesectoderm or ectomesenchyme, as opposed to those derived from the mesoderm. In amniote vertebrates, the mesectoderm is restricted to the cephalic NC whereas in the trunk, mesenchymal cells derive from the mesoderm (for references, Le Douarin and Kalcheim, 1999; Dupin et al., 2006). In contrast, in lower vertebrates, the NC not only forms the facial mesenchyme but also some trunk mesenchymal tissues, such as the distal bony rays of the fish caudal and dorsal fins. The NC origin of mesenchymal tissues was first mapped in quail-chick chimeras (Le Lièvre and Le Douarin, 1975; Couly et al., 1993) and, later, confirmed in mammals by genetic fate mapping using the *Cre-Lox* system associated with early NC-specific gene promoters (e.g., *Wnt1* and *Sox10*) (Chai et al., 2000; Jiang et al., 2000, 2002; Matsuoka et al., 2005; Gage et al., 2005). Taken together, these studies revealed the major contribution of the NC to the building of the vertebrate craniofacial skeleton and the formation of dermis, adipose tissue, tendons and connective components of muscles and glands in head and neck. In addition, the cephalic NCCs yield diverse ocular and dental tissues, and participate in cardio-vascular structures to yield the pericytes/smooth muscle cells lining the endothelium of forebrain blood vessels and of large arteries derived from aortic arches (Le Douarin and Kalcheim, 1999; Chai et al., 2000; Etchevers et al., 2001; Jiang et al., 2000, 2002). The diverse cell types derived from the NC are summarized in Table 1.

How such a broad NC lineage diversity is established in the developing embryo? Two main views have been explored: according to an early lineage determination model, NC-derived cell phenotypes differentiate from a heterogeneous collection of precursors, already committed in the pre-migratory NC. An alternative, stem cell-based view proposes that all the NC derivatives arise from a single type of multipotent stem cells similar to the production of all the distinct blood cells from the hematopoïetic stem cell. Disclosing the determination state of early NCCs could not been inferred from the behavior of cell populations and required the analysis of single NCC fate.

2. Evidence for NCC heterogeneity and multipotency in vivo

Quail-chick transplantations of the pre-migratory NC have been instrumental to establish the precise fate map of NC derivatives along the anterior-posterior axis. Moreover, further experiments of changing the position of NC sub-domains along this axis revealed that the fate of NCCs was not fixed in the neural primordium and rather depended upon local cues acting on NCCs during migration and at their sites of arrest (for references, Le Douarin and Kalcheim, 1999).

These transplantation data strongly supported the idea that NCCs were multipotent; however they could not exclude mosaïcism of the NC. Attempt to determine the time of NCC commitment in vivo was reached through the development of methods to mark single premigratory NCCs and follow their fate after their migration in early embryos. First sound evidence of NCC multipotency in vivo was put forward by tracing the progeny of individual cells labeled in the dorsal neural tube of the avian embryo following micro-injection with a vital fluorescent dye (Bronner-Fraser and Fraser, 1988, 1989). The progeny of about half of the labeled trunk NCCs populated more than one NC derivative and descendent cells adopted distinct fates, including glial cells, sensory and sympathetic neurons, melanocytes and

adrenomedullary cells. Multi-phenotypic clones were also found after the labeling of individual migratory NCCs (Fraser and Bronner-Fraser, 1991). The analysis of retrovirally-labeled NCCs homing to the chick dorsal root ganglia (DRG) also supported trunk NCC multipotency (Frank and Sanes, 1991). Bronner-Fraser and colleagues performed further experiments of intracellular dye injection into single pre-migratory trunk NCCs in the mouse (Serbedzija et al., 1994) and in Xenopus (Collazo et al., 1993), which were in general agreement with those results first obtained in the chick. Similar type of lineage tracing experiments in zebrafish, however have suggested that fate restrictions of NCCs may took place earlier in teleosts than in higher vertebrates (Raible and Eisen, 1994; Schilling and Kimmel, 1994). The clonal progeny of cranial NCCs contained only one type of differentiated cells (ganglion cells, chondrocytes or pigment cells) together with, in most cases, proliferating undifferentiated cells of unknown fate (Schilling and Kimmel, 1994). In the trunk, most of the NCCs contributed to a single derivative and about 20% generated a diversified progeny of pigment cells, glia and ganglionic cells (Raible and Eisen, 1994). Although providing evidence for early fate restrictions, these data are also indicative of the presence of (at least some) multipotent NCCs in the zebrafish NC.

These pioneer studies have revealed the heterogeneity of early NCCs with respect to their developmental fate in vivo. Some NCCs have multiple fates; others exhibit lineage restriction or differentiation bias already at pre-migratory stages. Kalcheim and collaborators recently investigated the cellular events that take place in the dorsal neural tube of the chick embryo (Krispin et al., 2010). After labeling tiny populations in the pre-migratory trunk NC at various times using vital dye microinjection or electroporation of a GFP reporter, they found that the fate of NCCs could be predicted from both their relative dorsal-ventral position in the neural tube and their time of delamination. Strikingly, the progeny of labeled NCCs populated a single derivative in nearly all cases and no labeled cells remained in the dorsal neural tube

(Krispin et al., 2010). These results support the idea of NC fate restriction prior to emigration, hence differ from previous data that had shown multipotency of pre-migratory trunk NCCs and the presence of dorsal neuroepithelial progenitors for both CNS and NC derivatives (Bronner-Fraser and Fraser, 1988). It is presently unclear whether these discrepancies are due to technical differences in the injection procedures (in the intact neural tube or using open-book neural tube preparations) or/and if they are related to variations of the stages and positions of the labeled dorsal neural tube cells/NCCs. One possibility is that in the early experiments, dye injection might have targeted NCCs at an earlier stage than in Krispin's study, before segregation between NC and neural tube lineages, although this issue needs to be resolved.

An important issue in the deciphering the mechanistics of NCC diversification is to ascertain when the fate of NC progenitors becomes irreversibly fixed. If migratory NCCs are multipotent, challenging them with distinct environments should be able to reverse fate restrictions imposed by the local cues. The influence of intrinsic and extrinsic factors in the regulation of NCC fate can be evaluated by grafting experiments. Baker et al. (1997) showed that, in heterochronic transplantations of the avian cranial NC, late-migrating cells can replace early-migrating ones and vice versa: both NCC populations were able to form melanocytes, neurons, glia, cartilages and bones in vivo (Baker et al., 1997). Therefore, the progressive ventral-to-dorsal pattern of colonization of the NC targets in normal development does not reflect distinct fate determination of early versus late, migratory NCCs. The environment thus plays a crucial role in the acquisition of NCC fate, which however does not exclude that NCC populations along the AP axis do not possess equivalent differentiation potentials (Nakamura and Lelièvre, 1982; McKeown et al., 2003; Lwigale et al 2004).

3. In vitro clonal cultures to characterize NCC developmental potentials

Similar to the in vitro assays for colony-forming units devised in the 1950ies onward to decipher the origin of hemopoïetic cell diversity, several laboratories have undertaken in vitro cultures of single NCCs in avian and mammalian species. The in vitro clonal approach is aimed at defining the whole spectrum of developmental options that a NCC can express; its goal is thus slightly different from NCC lineage tracing in vivo, which follows the actual differentiation fate of NCC progeny in embryogenesis. The in vitro clonal studies also permitted to challenge NCC differentiation options in order to characterize the influences of extracellular factors on the behavior of specific sets of NC progenitors.

This type of work, pioneered by Cohen and Konigsberg (1975), identified a common progenitor for pigmented melanocytes and unpigmented cells in the avian trunk NC. Accordingly, these authors devised a method to isolate early NCCs as a pure population, when they have migrated away from an explanted neural primordium and form an outgrowth of adherent cells on the culture substrate (Figure 1). This method was later adapted to isolate the NCCs (or subsets of them) from diverse levels of the neural axis in different species (Etchevers, 2011). Moreover, modified culture conditions and phenotype-specific markers also improved, which allowed more extensive analysis of the developmental differentiation capacities of single NCCs from different sources (Crane and Trainor, 2006; Dupin et al., 2006; Delfino-Machin et al., 2007 for references).

The classical in vitro clonal analysis of avian NCCs performed by Sieber-Blum and coll. and Le Douarin and coll. (Baroffio et al., 1988, 1991; Dupin et al., 1990; Lahav et al., 1998; Sieber-Blum, 1989, 1991; Sieber-Blum and Cohen, 1980; Sieber-Blum et al., 1993) provided compelling evidence for the presence of several types of multipotent (able to yield various combinations of three or more NC derivatives) as well as more restricted progenitors in te population of early NCCs. Trunk NC multipotent cells were able to give rise to pigment cells,

myofibroblasts/smooth muscle cells and PNS glial cells and neurons, thus recapitulating the full repertoire of trunk NC derivatives (Trentin et al., 2004).

Experiments performed on early migratory NCCs isolated from the quail mesencephalon highlighted the heterogeneity of early cranial NCCs regarding their proliferation and differentiation capacities (Baroffio et al., 1988, 1991; Dupin et al., 1990; Trentin et al., 2004). The majority of clonogenic cranial NCCs generated a progeny with diverse combinations of two to four distinct cell phenotypes. NCC subsets thus yielded colonies including myofibroblasts and/or chondrocytes together with neuronal, glial and melanocytic cells, therefore providing first evidence that the segregation of "neural-melanocytic" and "mesenchymal" lineages was not completed at the onset of NCC migration. Moreover, intermediate progenitors in the mesencephalic NC, such as glial-melanocytic and glial-myofibroblastic bipotent ones, exhibited stem cell properties as they could self-renew along successive rounds of in vitro subcloning (Trentin et al., 2004).

In the quail (Ito and Sieber-Blum, 1991) and mouse (Youn et al., 2003), the "cardiac" NCCs also include common progenitors for neurons, pigment cells, fibroblasts and chondrocytes. These "cardiac" NCCs emerge from the posterior rhombencephalon and colonize the cardiac outflow tract where they play an important function in heart septation (Kirby and Waldo, 1995).

The in vitro culture methods of avian NCCs adapted to mammalian cells likewise led to identify multipotent progenitors in the mouse and rat trunk NC (Ito et al., 1993; Stemple and Anderson, 1992; Shah et al., 1994, 1996). Precursors isolated in the rodent NC by means of expression of p75 receptor, gave rise to autonomic neurons, glial cells and myofibroblasts and exhibited self-renewal in vitro, thus deserving to be considered as NCSCs (Stemple and Anderson, 1992).

Importantly, NCSCs were found not only in the early NCC population but also in several NC

derivatives, including peripheral nerves, gut and DRG, as well as in a plethora of locations in adult mammalian tissues (for references, Shakhova and Sommer, 2010). The isolation and properties of these postmigratory and adult NCSC-like progenitors are presented in the next sections 8-9 of this review.

4. Melanocytic, neural and mesenchymal derivatives of the cephalic NC arise from common progenitors

The large diversity of cell types originating from cranial, as compared with trunk NCCs, had suggested that the mesenchymal lineages could be segregated from the other, trunk NC-like, lineages (i.e., melanocytes and PNS cells) in the premigratory cranial NC. Single cell in vitro analysis offered the opportunity to explore whether the mesenchymal cranial NC derivatives (e.g., smooth muscle cells, chondrocytes, osteocytes and adipocytes) arose either from multipotent NCCs or from distinct, independent NC progenitors.

Clonogenic cells able to yield nearly all of the phenotypes normally derived from the cephalic NC, including the skeletal ones, were evidenced recently (Calloni et al., 2007, 2009; Le Douarin et al., 2008). Chondrocytes differentiated and formed aggregates in about 15% of the colonies obtained from quail mesencephalic-rhombencephalic NCCs harvested after a 15-hour period of migration from the neural primordium. Nearly all of the chondrogenic progenitors were multipotent and gave rise, in addition to chondrocytes, to glia, melanocytes, myofibroblasts and/or neurons (Calloni et al., 2007). In vivo, cephalic NCCs yield the craniofacial bones that differentiate along either endochondral or dermal (membranous) ossification (Couly et al., 1993; Helms and Schneider, 2003; Minoux and Rijli, 2010). Accordingly, two types of osteoblasts developed in quail NC cultures: endochondral-like osteoblasts in the perichondrium of cartilage nodules, and dermal-like osteoblasts, which clustered independently of the presence of chondrocytes (Calloni et al., 2009). Unexpectedly,

more than 90% of the colonies contained osteoblasts (which expressed *Runx2*, the early marker gene of the bone cell lineage) (Ducy et al., 1997), together with one or several other NC-derived cell types. Among these very diverse types of progenitors, a highly multipotent NCC showed the ability to generate all the recorded phenotypes, i.e., glia, autonomic neurons, melanocytes, myofibroblasts, chondrocytes and osteocytes (Calloni et al., 2009; Dupin et al., 2010) (Figure 2). This multipotent progenitor (termed GNMFCO) lies upstream of various distinct NCCs endowed with more restricted developmental options, consistent with a hierarchical model of NC lineage diversification, in which environmental signals impart progressive fate restrictions to multipotent stem cells. Although its self-renewal property still deserves to be demonstrated, the highly multipotent GNMFCO progenitor recorded in the cephalic quail NC might represent the NCSC capable of generating most (if not all) the NC-derived cell types (both neural-melanocytic and mesenchymal/skeletogenic), which concur in building the head of amniote vertebrates (Dupin et al., 2010).

5. Mesenchymal differentiation capacities in the trunk NC

As opposed to cephalic NCCs, the fate repertoire of trunk NCCs in amniote vertebrates is limited to PNS cells, melanocytes and adrenomedullary cells. The unique and very small, mesenchymal contribution of the trunk NC seems to be endoneurial fibroblasts of the sciatic nerve, which derive, together with Schwann cells, from NCSCs expressing *Desert Hedgehog* in transgenic mice (Joseph et al., 2004).

From emerging recent data, it is now clear that trunk NCCs in amniotes do have the capacity to give rise to diverse mesenchymal phenotypes, including skeletal cells, which can be disclosed upon specific conditions in vitro and in vivo. In appropriate culture conditions, trunk NCCs could differentiate into adipocytes (Billon et al., 2007) and chondrocytes (McGonnell and Graham, 2002; Abzhanov et al., 2003; Ido and Ito, 2006; Calloni et al.,

2007). Similar to cephalic NCCs, in vitro chondrogenesis by avian trunk NCCs markedly increased following exposure to Sonic Hedgehog (Shh). However, the rate of chondrogenesis was much lower in trunk than cranial NC cultures (Calloni et al., 2007; Le Douarin et al., 2008). Hence in vitro clonal assay led to identify a rare (1/225) chondrogenic progenitor in the quail trunk NC, which produced glial cells, myofibroblasts and chondrocytes (Calloni et al., 2007).

Together with recent evidence that avian trunk NCCs can differentiate in vitro into osteoblasts (McGonnell and Graham, 2002; Dupin, Coelho and Le Douarin, unpublished data), these findings indicate that, albeit reduced, mesenchymal potentials are present in the trunk NC of amniotes (Figure 3). Importantly, in vitro clonal data suggested that mesenchymal phenotypes did not arise from independent precursors in both cranial and trunk NCCs (Calloni et al., 2007, 2009). One attractive possibility is that multipotent neural-melanocytic-mesenchymal progenitors recorded in the avian NC might represent a primitive type of NCC. In early vertebrates like fossil fishes (Ostracoderms) of the Ordovician, the head and trunk was covered by a superficial armor of calcified dermal bone, which is assumed to derive from the NC since it contained dentine (Janvier, 1996; Smith and Hall, 1990; Smith, 1991). During evolution, this superficial skeleton regressed in the trunk region while substituted for by an internal endochondral skeleton of mesodermal origin. The dermal skeleton in the distal part of the fin rays of teleosts, which derives from the trunk NC (Smith et al., 1994), may be considered as a remnant of the primitive superficial skeleton of extinct vertebrates. In higher vertebrates, NC skeletogenic fate has been retained only in the craniofacial region, whereas it vanished, but did not completely disappeared, in the trunk.

6. Environmental cues regulating lineage diversification of NC progenitors in vitro

The crucial influence of environmental factors on the differentiation of NCC populations is

recognized for long. The role of transcription factors and signaling pathways in NC phenotype specification has been reviewed elsewhere (see Sommer, 2006 and Raible and Pavan, this issue). Here we summarize a number of in vitro culture studies that uncovered the action of environmental signals on specific types of NC progenitors.

In mammals, Anderson and co-workers have identified several molecular pathways involved in the differentiation choice of trunk NCSCs that produce glia, autonomic neurons and myofibroblasts. BMP2 signaling promoted neuronal autonomic outcome at the expense of glial cell fate (Shah et al., 1996) whereas Neuregulin-1 (Shah et al., 1994) and Delta-mediated Notch receptor activation (Morrison et al., 2000) triggered acquisition of a glial fate. Developing mammalian NCSCs also use TGFβ reiteratively. This factor instructs multipotent NCCs to adopt a myofibroblast fate (Shah et al., 1996) and it regulates NCC apoptosis (Hagedorn et al., 1999; 2000). Moreover, TGFβ promotes the acquisition of mesenchymal fate while suppressing neurogenic potential of early NCCs (John et al., 2011).

Signals that drive multipotent NCCs to form sensory neurons involve activation of the Wnt/β-catenin canonical signaling pathway, which is not only required but also sufficient to trigger sensory neurogenesis in vivo (Lee et al., 2004). Moreover, Wnt factors together with BMPs ensure the maintenance of multipotent mouse NCSCs (Kleber et al., 2005). These findings as well as the role of Wnt signaling in melanocyte development are discussed in detail elsewhere (Sommer, 2006 and Sommer, 2011).

Sonic hedgehog (Shh) plays a crucial role in the development of cephalic NC progenitors. The early NCC survival in the branchial arches and later, facial morphogenesis, require signaling by Shh, which is produced by the pharyngeal endoderm when migratory cranial NCCs invade branchial arches (Ahlgren and Bronner-Fraser, 1999; Brito et al., 2006; Jeong et al., 2004; Wada et al., 2005). In vitro culture experiments have shown that Shh short- term treatment influenced the behavior of cephalic quail NC progenitors: Shh strongly promoted

NCC chondrocytic fate and enhanced the development of multipotent progenitors endowed with both neural-melanocytic and mesenchymal (including chondrocytic) differentiation potentials (Calloni et al., 2007; Le Douarin et al., 2008). Notably, quantification of Shh-treated and untreated clones indicated that increase in the "mesenchymal-neural" progenitors was coincident with decreased ratio of the "only-neural" ones, suggesting that Shh can maintain NCC multipotentiality. Likewise, Shh significantly raised the frequency of highly multipotent GNMFCO progenitors while not changing the overall frequency of osteogenic NCCs (Calloni et al., 2009; Dupin et al., 2010). These findings suggest that Shh favors the development of multipotent cephalic NCCs by promoting mesenchymal fates while it does not significantly alter their differentiation along the melanocytic and neural phenotypes.

The vasoactive peptide endothelin-3 (EDN3) is one of the important environmental factors in the regulation of melanocytic differentiation (for references, Dupin and Le Douarin, 2003; Thomas and Erickson, 2008; Saldana-Caboverde and Kos, 2010). Mouse genetics has revealed that signaling by EDN3 and its receptor at early NC developmental stages was required cell-autonomously for proper pigmentation and enteric neurogenesis (for reviews, Gershon, 1999; Saldana-Caboverde and Kos, 2010). EDN3 also influenced the development of avian melanocyte progenitors in vitro (Lahav et al., 1996, 1998; Trentin et al., 2004). At early migratory stages, EDN3 showed a strong mitogenic effect on avian NCCs in culture. In clonal NC cultures, the specific target cells of EDN3 turned out to be bipotent glial-melanocytic NCSCs, whose survival, proliferation and self-renewal were promoted in the presence of EDN3 (Lahav et al., 1998; Trentin et al., 2004). Other types of intermediate progenitors such as glial-myofibroblast precursors were not responsive to this factor.

7. The dual origin of melanocytes and NC glial-melanocyte lineage relationships

The clonal assays mentioned above indicated the presence of cells in the avian NC that are both multipotent upon isolation and multifated in vivo. These cells were found concomitantly with more lineage-restricted cells, raising the question of when lineage segregations occur during NC development. This question is of particular interest with respect to the specification of the melanocyte lineage.

Indeed, recent studies suggest a dual origin of melanocytes from NC or NC-derived cells. According to the traditional model, the melanocyte lineage segregates at early stages of NC development, i.e., soon after NCCs have emigrated from the neural tube. Unlike neural progenitors that migrate ventrally, melanocyte progenitor cells engage in a dorsolateral pathway underneath the ectoderm after having stalled for a short period in the so-called "migration staging area" close to the neural tube (Weston, 1991). In avian and zebrafish embryos, ventral migration occurs before dorsolateral migration (Erickson et al., 1992) (Raible et al., 1992), and this temporal migration pattern appears to coincide with melanocyte specification (Henion and Weston, 1997). As mentioned before, recent work by Kalcheim and colleagues further supports a temporal control mechanism of lineage segregation in the avian NC (Krispin et al., 2010), whereby early emigrating NCCs are restricted to neural lineages, while late-migrating NCCs only produce melanocytes, but no neural cells. In contrast, earlier reports (Bronner-Fraser and Fraser, 1988, 1989; Frank and Sanes, 1991), suggested that at least some pigment cells do not originate from lineage-restricted but rather from multipotent NCCs. This might also be the case in mice, where unlike in avian embryos, NCCs migrate along the dorsolateral pathway concomitantly with ventral migration already at early stages of NC development (Serbedzija et al., 1990). Indeed, dye labeling of individual NCCs revealed multiple clones with daughter cells localized to ventral neural structures and to the dorsolateral pathway (Serbedzija et al., 1994). This does not exclude that, as in chicken, neural and melanocyte fate decisions are subject to temporal control mechanisms in mice, too.

Compatible with this idea are results obtained by genetic manipulation of *β-Catenin*, an essential component of canonical Wnt signaling: Conditional *β-Catenin* inactivation in premigratory NCCs abolishes both sensory neuron and melanocyte formation (Hari et al., 2002). In contrast, constitutive activation of *β-Catenin* in the premigratory NC promotes sensory neurogenesis at the expense of virtually all other NC derivatives, including melanocytes (Lee et al., 2004), suggesting that the sensory lineage choice occurs before Wnt/β-Catenin-dependent melanocyte generation. However, to assess the extent of melanocyte formation by early versus late emigrating NCCs in the rodent NC, it will be necessary to complement the original clonal assays (Serbedzija et al., 1994) by single cell fate mapping experiments in vivo using appropriate genetic tools (Livet et al., 2007) (Snippert et al., 2010).

Apart from the traditional view of melanocyte generation involving dorsolaterally migrating neural crest cells, melanocyte formation has also been associated recently with cells migrating first ventrally and then along nerves (Adameyko et al., 2009) (Figure 4). Tracking of neural tube cells by green fluorescent protein (GFP) expression in chicken, revealed NC-derived cells localizing to the distal ends of nerves in the skin and apparently acquiring a melanocyte fate after they detach from the nerves. A large fraction of melanocytes in the skin actually derived from nerve-associated cells rather than from NCCs migrating along the dorsolateral pathway, which differentiate into melanocytes earlier.

These data were also supported by Cre/LoxP-based genetic lineage mapping of glial precursors along nerves of mouse embryos, which pointed to a time window around embryonic day (E)11, when nerve-cells produce cells expressing melanocyte markers (Adameyko et al., 2009) (Figure 4). The numbers of melanocytes in the adult skin originating from nerve cells might be difficult to estimate, however, as the Cre line used in these studies

displays transient expression in multipotent neural crest cells at early stages and in at least some dorsolateral melanocytes (Leone et al., 2003).

Although melanocyte formation from nerve cells appears to be restricted normally to early developmental stages, cells present in nerves have the potential to produce melanocytes also after birth. When cultured in medium permissive for melanocyte formation, Schwann cells de-differentiate into a glial-melanocytic progenitor able to give rise to pigmented cells (Sherman et al., 1993) (Dupin et al., 2003). Moreover, sciatic nerve injury leads to pigment formation around damaged nerves (Rizvi et al., 2002) (Adameyko et al., 2009). However, the physiological relevance of this process is unknown, and it remains to be addressed whether in vivo, melanocytes generated from developing or injured nerves originate from bipotent Schwann cell precursors, from de-differentiating Schwann cells, from lineage-restricted melanoblasts potentially present in nerves along with Schwann cell precursors as a discrete cell population, or even from multipotent NCSCs present in nerves.

Noticeably, the reverse transition, from melanocytes to glial cells, could be observed in single cell cultures of pigment cells isolated from the quail epidermis, in which melanocytes reverted to multipotent and self-renewing NC-like progenitors (Dupin et al., 2000; Real et al., 2006). As shown recently, mammalian melanoblasts and melanocytes likewise are prone to erase lineage restrictions in vitro and dedifferentiate into immature NCSCs (Motohashi et al., 2009; Zabierowski et al., 2011).

These findings, which unravel the high plasticity and mutual relationships of the glial and melanocytic NC lineages, open for the identification of new factors and the reevaluation of the mechanisms regulating glial and pigment cell differentiation.

8. Postmigratory NCSCs

The idea that nerve-derived melanocytes originate from multipotent cells is not far fetched. Indeed, cells with NCSC properties have by now been isolated from most if not all NC target structures, including the sciatic nerve, the nerves innervating the skin and cranial nerve terminals within the palatal ridges (Delfino-Machin et al., 2007; Shakhova and Sommer, 2010; Widera et al., 2011) (Table 2). Surprisingly, not only fetal but also adult tissues appear to contain NC-derived cells that express NCSC markers, are multipotent, and are able to self-renew comparable to their embryonic counterparts. It has to be noted, however, that postmigratory NCSCs isolated from different regions or at different time points are intrinsically different, exhibiting differential responses to microenvironmental signals (Bixby et al., 2002; Wong et al., 2006). These differences can affect differentiation, with NCSCs being more or less neurogenic depending on the spatiotemporal context (White et al., 2001; Mosher et al., 2007). Differences among NCSC populations have also been observed with respect to their self-renewal capacity and growth requirements. At early developmental stages, combinatorial Wnt and BMP signaling regulates self-renewal of migratory NCSCs (Kleber et al., 2005). Once NCSCs have reached their targets, however, responsiveness to Wnt/BMP is lost and proliferation is regulated by an EGF/small RhoGTPase signaling cascade (Fuchs et al., 2009). Such intrinsic changes in behavior and factor responsiveness are thought to allow NCSCs to adapt to a dynamically changing microenvironment and to produce the cell types appropriate for the location and developmental stage (Falk and Sommer, 2009).

The first description of postmigratory NCSCs goes back to 1999, when Morrison and colleagues prospectively isolated cells with stem cell features from rat E14.5 sciatic nerve by flow cytometry using antibodies against the low affinity neurotrophin receptor $p75^{NTR}$ (Morrison et al., 1999) (Table 2). Nerve-derived, $p75^{NTR}$-expressing cells displayed self-

renewal potential *in vitro* and *in vivo* and produced neurons and glia upon transplantation into chick embryos. Conceivably, the neuronal potential of these cells can only be revealed upon transplantation and might not reflect an actual in vivo fate. However, cellular tracking experiments in mouse embryos demonstrated that some fetal sciatic nerve cells are at least bipotent, generating glia and endoneurial fibroblasts in vivo (Joseph et al., 2004).

Similar to sciatic nerves, both the fetal and the adult enteric nervous system (ENS) were shown to contain multipotent $p75^{NTR}$-positive cells with self-renewing potential (Bixby et al., 2002; Kruger et al., 2002). However, as shown by BrdU incorporation experiments, the self-renewal capacity of these cells decreases with age. Moreover, the differentiation potential of adult enteric NCSCs differs substantially from the one of fetal NCSCs, with adult cells being rather gliogenic than neurogenic. Although not directly demonstrated, these NCSCs might be implicated in the de novo formation of neurons, which has been observed by Gershon and colleagues in the adult mouse gut after treatment with serotonin (Liu et al., 2009). This study revealed an unexpected degree of plasticity in the ENS and pointed to the exciting possibility that serotonin agonists might be used in the future to promote survival and formation of enteric neurons in patients suffering from ENS disorders.

De novo neurogenesis has also been found to occur in sensory ganglia upon capsaicin-mediated neuronal cell death (Czaja et al., 2008). Again, it is conceivable that neurogenesis in such ganglia might involve injury-induced activation of resident cells with stem cell features. Cells with an unexpected developmental potential have first been identified in cranial nodose sensory ganglia and dorsal root ganglia (DRG) in the course of transplantation experiments in avian embryos (Ayer-Le Lievre and Le Douarin, 1982; Schweizer et al., 1983). Later, multipotent cells with self-renewal capacity were isolated from rodent DRG during fetal development (Hagedorn et al., 1999; Hjerling-Leffler et al., 2005) and from adult tissue (Li et al., 2007; Nagoshi et al., 2008), although cells derived from adult DRG exhibited only a

limited self-renewal potential (Table 2). DRG axotomy combined with BrdU incorporation assays revealed that neurons were BrdU-negative (i.e. not induced to proliferate upon injury), whereas many cells surrounding neurons were BrdU-positive, suggesting an association of DRG-derived progenitors with the glial lineage (Li et al., 2007).

The capacity of glial cells to re-initiate proliferation and to adopt progenitor features has also been demonstrated for the carotid body (CB), an oxygen-sensing organ of the sympathoadrenal lineage derived from the NC (Pearse et al., 1973). Under low-oxygen conditions, a stem cell-like population of the CB proliferates and gives rise to new dopaminergic neurons in vivo and in vitro (Pardal et al., 2007). Genetic cell fate mapping by means of a Wnt1-Cre reporter line demonstrated that these CB stem cells are glial cells with self-renewal capacity and the potential to produce tyrosine hydroxylase-positive neurons and non-neural cells in vitro. When activated by hypoxia in vivo, the glial marker GFAP is downregulated and the cells become proliferative intermediate progenitors that contribute to de novo neurogenesis. These CB stem cells are, however, not always active. Rather, on return to normoxia, GFAP is re-expressed and the cells become quiescent again. These results are important for the field, as they point for the first and so far, only time, to a potential physiological role of NCSCs in the adult organism.

For many other NC target tissues, the functional role of resident cells with NCSC potential remains to be elucidated. This is the case, for instance, for cells isolated from the adult cornea, which is partially composed of NC-derived tissues (Creuzet et al., 2005; Gage et al., 2005; Ittner et al., 2005; Johnston et al., 1979). Cornea-derived cells expressing various NC markers can differentiate into neurons, adipocytes, chondrocytes, and osteoblasts, although the overall potential of these cells and their exact origin in the anterior eye compartment is unclear (Yoshida et al., 2006; Brandl et al., 2009; Shakhova and Sommer 2010).

Many other craniofacial tissues, such as the nasal mucosa, the palatum ridges, the olfactory and respiratory mucosa are likely to comprise multipotent stem cells of NC origin, as suggested by recent reports (for references, Klaschmidt et al., 2011).

A potential reminiscent of NCCs was also discovered by Shi and collaborators in cells derived of the dental mesenchyme, isolated from adult and postnatal dental pulp of different species, and from human peridondal ligament (Gronthos et al., 2000, 2002; Miura et al., 2003; Seo et al., 2004). Thereafter, a growing number of investigators explored the differentiation properties of NC-like progenitors from human dental tissues, which hold promise for tooth regeneration and stem cell-based therapy (for review, Volponi et al., 2010).

Furthermore, NCSC-like cells have been obtained from neonatal and adult heart (El-Helou et al., 2008; Tomita et al., 2005). In vitro, these nestin-positive cells were able to form self-renewing spheres that could differentiate into neural and smooth muscle cells and migrate to the cardiac outflow tract and structures of the PNS when transplanted into chicken embryos (Tomita et al., 2005). Intriguingly, infarct regions of rat and human hearts also contain sphere-forming nestin-expressing cells, although their NC origin remains to be established. These cells have apparently the capacity to invade rat infarct regions and to contribute to reparative fibrosis in the injured area (El-Helou et al., 2008).

The potential of bone marrow stromal cells (BMSCs) to differentiate into mesenchymal cell types, such as osteoblasts, chondrocytes, adipocytes, and myoblasts, has been known for a long time. Surprisingly, BMSCs were also reported to generate neurons and glia in cell culture and upon transplantation into the CNS (Arnhold et al., 2006; Azizi et al., 1998; Hofstetter et al., 2002; Sanchez-Ramos et al., 2000; Woodbury et al., 2000). Conceivably, the neural potential of BMSCs is due to the presence of a NC-derived cell subpopulation in bone marrow. Indeed, genetic in vivo fate mapping using *Wnt1-* and *P0*-promoter driven expression of *Cre* reporter alleles in mice, allowed the identification of NC-derived cells in the bone

marrow (Nagoshi et al., 2008). These cells could be propagated in cultures of spheres, most of which were developmentally restricted to mesenchymal fates. However, a small percentage of isolated bone marrow cells had the capacity to generate neurons, glia, and smooth muscle cells. Collagenase treatment increased the number of NCSC-like cells that could be obtained from bone marrow, indicating a tight association of these cells with the bone marrow surface. Thus, the bone marrow appears to contain distinct stem cell types of different developmental origins, including hematopoietic stem cells (HSCs), mesenchymal stem cells, and NCSCs. It is a fascinating thought that these different stem cell types might functionally interact with each other, for instance by mutually providing niches allowing the coordinated regulation of tissue growth and homeostasis. In support of this model, nestin-positive cells in the bone marrow have recently been demonstrated to serve as niche for HSCs (Mendez-Ferrer et al., 2010). Indeed, NC-derived non-myelinating Schwann cells, located along sympathetic nerve terminals in the bone marrow maintain HSC via TGFβ signaling (Yamazaki et al., 2011).

9. Multipotent NC-like progenitors in the skin

The skin is another organ harboring multiple stem cell types. In particular, hair follicles and closely associated structures contain epidermal stem cells, dermal stem cells, muscle stem cells, and cells with NCSC features. In vitro, cells from human, pig, and rodent skin turned out to be multipotent and able to generate neurons, glia, melanocytes, smooth muscle cells, chondrocytes, osteoblasts, and adipocytes (Toma et al., 2001, 2005; Belicchi et al., 2004; Dyce et al., 2004; Joannides et al., 2004; Fernandes et al., 2004; Sieber-Blum et al., 2004; Amoh et al., 2005; Shih et al., 2005; Wong et al., 2006; Clewes et al., 2011). Freda Miller's group pioneered this work and coined the term skin-derived precursor (SKP) for multipotent cells isolated from facial and trunk skin of rodents and human subjects, and enriched based on their sphere-forming capacity (Toma et al., 2001; Fernandes et al., 2004). Other groups

isolated and expanded multipotent cells from the skin based on expression of HSC markers (Belicchi et al., 2004) or based on GFP expression driven from *nestin* promoter elements in transgenic animals (Amoh et al., 2005). However, these studies left open the origin of multipotent skin cells. SKPs express NC markers, such as Slug, Snail, Twist, Pax3, and Sox9, consistent with a NC origin. Intriguingly, however, when EYFP-labeled SKPs were transplanted into the path of migratory NC in chicken embryos, some migrated to NC target structures, but others engrafted to the dermal layer of the skin without apparent association with NC derivatives. These findings suggested that SKPs might either be NC-derived and able to transdifferentiate into non-NCC types or that SKPs might represent more than one discrete cell populations originating from distinct skin structures.

This issue was later addressed by genetic fate mapping using *Wnt1-Cre/R26R* mice to trace NCC lineages, which surprisingly demonstrated multiple origins of multipotent skin cells (Table 3). In the facial skin, where the mesenchyme derives from the NC, cells with neural and non-neural potentials have been associated with the hair follicle bulge and the dermal papilla (Fernandes et al., 2004; Sieber-Blum et al., 2004). Subsequently, in vivo fate mapping experiments combined with microdissection of whisker follicles demonstrated the presence of sphere-forming cells within multiple NC-derived mesenchymal structures, including the capsula, the dermal sheath, and the ringwulst of whisker follicles (Wong et al., 2006). Thus, adult NCSCs are not confined to a particular niche in the facial skin, but can be obtained from all NC-derived tissues analyzed (Table 3).

Multipotent cells exhibiting NCSC features have also been isolated from trunk skin, where the mesenchyme originates from the mesoderm rather than the NC. Freda Miller's group has reported the derivation of SKPs from juvenile and adult mouse trunk skin that could be extensively passaged in culture and forced to differentiate into cell types as diverse as neurons, glia, smooth muscle, and adipocytes (Toma et al., 2001). Based on the finding that

the dermal papilla of whisker follicles represented a structure harboring SKPs from facial skin (Fernandes et al., 2004), it has been suggested that SKPs from the trunk might also be associated with dermal papilla from hair follicles. If so, SKPs would have distinct origins depending on their source (NC-derived in facial skin vs. mesoderm-derived in trunk skin). In accordance with a potential mesodermal origin, SKPs were reported to be negative for the NCSC marker $p75^{NTR}$ and to exhibit a molecular signature distinct from that of actual NCSCs (Hu et al., 2006). Furthermore, SKPs have been isolated from mice carrying a *Sox2-GFP* transgene, which is strongly expressed in the dermal papilla of anagen follicles in the trunk (Biernaskie et al., 2009). In this study, FACS-isolated, GFP-positive cells were shown to regulate hair follicle morphogenesis and to localize to reconstituted dermal papilla, dermal sheath, and dermis, thus exhibiting dermal stem cell features. In addition, Sox2-positive cells were spherogenic, multipotent in vitro, and partially able to localize to nerves and DRG after transplantation into the NC migratory stream of chicken embryos.

These data are consistent with the hypothesis that trunk SKPs derive from a mesodermal structure, the dermal papilla, but are able to acquire NCSC features upon culturing. A mesodermal origin of SKPs with the capacity to generate cell types normally arising from the NC has recently been supported by fate mapping of SKPs using a *Myf-Cre* allele, which targets cells developing from somites (Jinno et al., 2010). However, it cannot be excluded that Sox2-positive stem cells can also originate from trunk NC derivatives, given the expression of Sox2 in several NC lineages. Indeed, multipotent sphere-forming cells expressing the NCSC markers $p75^{NTR}$ and Sox10 have been shown by in vivo fate mapping using various Cre-reporter lines to be NC-derived and to be associated with the glial and melanocytic lineages in the hair follicle bulge in trunk skin (Wong et al., 2006). Intriguingly, cells with sphere-forming potential could not be isolated from sciatic nerves, demonstrating that nerves or nerve endings in the skin, but not peripheral nerves in general, contain cells with stem cell

properties. In sum, the skin appears to harbor different cell types displaying multipotency and self-renewal capacity upon isolation (Table 3).

10. Conclusive remarks and perspective

Although debate still exists as to whether most individual NCCs or only a small subset of them are multipotent after emigration from the neural primordium, a large body of evidence demonstrated the multipotency and self-renewal capacity of various subsets of NC progenitors in avian and mammalian species. Taken together, the analysis of NC clonal progeny in vivo and in vitro have disclosed certain heterogeneity of the early NCCs with respect to their developmental potentials, with the presence of both multipotent cells and more restricted progenitors. The cephalic NC contains stem/progenitor cells of a very wide differentiation repertoire, encompassing neural, melanocytic and various mesenchymal phenotypes. Such diversity of differentiation options is unique among the other somatic progenitors found in higher vertebrates, and led some authors to consider the NC as a putative fourth germ layer (Hall, 2000).

The evidence for the multipotency of NCCs and the phenotypic plasticity of some of the NC-derived cells is mostly based upon the analysis of the developmental repertoire that individual early NCCs and NC-derived cells displayed in vitro. Due to technical limitations, in vivo lineage tracing approaches so far provided few and contradictory results regarding lineage determination in single NCCs. The next challenges will be to complement the in vitro work by NC lineage tracing in vivo in different species. New methods to permanently trace distinct subpopulations of individual cells in avian (Sato et al., 2007; Yokota et al., 2011) and rodent (Livet et, 2007; Snippert et al., 2010; Gerrits et al., 2010; Beier et al, 2011) embryos, would help gaining further insights into the cellular mechanisms of NC lineage segregation in vivo.

A number of recent reports showed that even the adult vertebrate organism contains cells that exhibit properties of multipotent NCSCs, at least upon isolation. Such cells were also traced to tissues such as the bone marrow that before the advent of genetic fate mapping techniques in the mouse were not known to contain NC-derived cells (Nagoshi et al., 2008; Takashima et al., 2007). These unexpected findings raise the question of whether NCSCs in the bone marrow might account for the neural potential, sometimes observed in cultures of mesenchymal stem cell preparations. In vivo lineage tracing also revealed that SKPs originating from the mesoderm rather from the NC display striking similarities to NCSCs with respect to their self-renewing capacity and developmental potential (Biernaskie et al., 2009). It remains to be shown whether the similar behavior of mesoderm- and NC-derived cells might reflect shared intrinsic developmental programs or might be due to reprogramming events taking place in culture.

Because of their broad potential and accessibility, NCSC-like cells from adult tissues have also been implicated as potential sources for future cell and tissue replacement therapies. For instance, both SKPs and EPI-NCSCs had the capacity to generate myelinating glia in models of spinal cord or peripheral nerve injuries (Sieber-Blum et al., 2006; McKenzie et al., 2006; Biernaskie et al., 2007). Moreover, SKPs were reported to produce osteocytes and chondrocytes functionally integrating into bone tissue *in vivo* (Lavoie et al., 2009). Because the developmental and therapeutic potentials of stem cells likely depend on their nature and origin, it will be important to better characterize distinct populations of adult NCSC-like cells and to investigate the mechanisms controlling their fate *in vitro* and *in vivo*. These endeavors will also help to determine the physiological roles of adult NCSCs and to gain insights into

pathophysiological processes possibly involving these cells, such as the initiation and propagation of melanoma and other tumors with a NC origin.

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Figure Legends

Figure 1: Method of in vitro clonal cultures of avian NCCs.

(1) Migratory NCCs are harvested from primary cultures of neural tube explants isolated at cephalic and trunk levels from quail embryos at 7-somite-stage (SS) and 20SS, respectively. (2) Single NCCs are then individually plated under microscopic control on a feeder-layer of growth-arrested 3T3 fibroblasts and cultured in condition medium appropriate for the differentiation of main NC-derived lineages. (3) After 8 to 10 days, the differentiated cell types in the colonies are identified using phenotype-specific markers in order to determine the developmental potentials displayed by the founder NCCs.

Figure 2: Schematic representation of the progenitor types identified by in vitro clonal analysis of quail cephalic NCCs.

The progenitors are classified according to the number of cell phenotypes recorded in the clones following analysis of glial cells (G), neurons (N), melanocytes (M), myofibroblasts (F), chondrocytes (C) and osteoblasts (O). The percentage of each progenitor type is indicated. The great majority of clonogenic NCCs produced osteoblasts and generated both neural/melanocytic and mesenchymal cell types (progenitors in grey). Smaller subsets of precursors yielded neural/melanocytic cells (G, N, M) only (in yellow) or mesenchymal cell types only (F, C, O) (in blue). A highly multipotent GNMFCO progenitor is upstream in the hierarchy of cephalic NC progenitors. Adapted from Calloni et al., (2009).

Figure 3: Melanocyte specification from NCCs.

Melanocytes are either directly generated from the NC (in the mouse at approx. E9) (A, B) or indirectly from nerve cells (in the mouse, during a time window around E11) (C). While some studies favor the view that melanocyte specification occurs in multipotent NCSCs (e.g. once these cells have reached the migration staging area (MSA) adjacent to the neural tube) (A), other reports propose that lineage segregation takes place already in premigratory NC cells (B). A second wave of melanocytes derives from nerve cells, but it remains unclear whether this involves differentiation of multipotent NCSCs present along the nerves or the conversion of Schwann cell precursors (or Schwann cells) to melanocytes.

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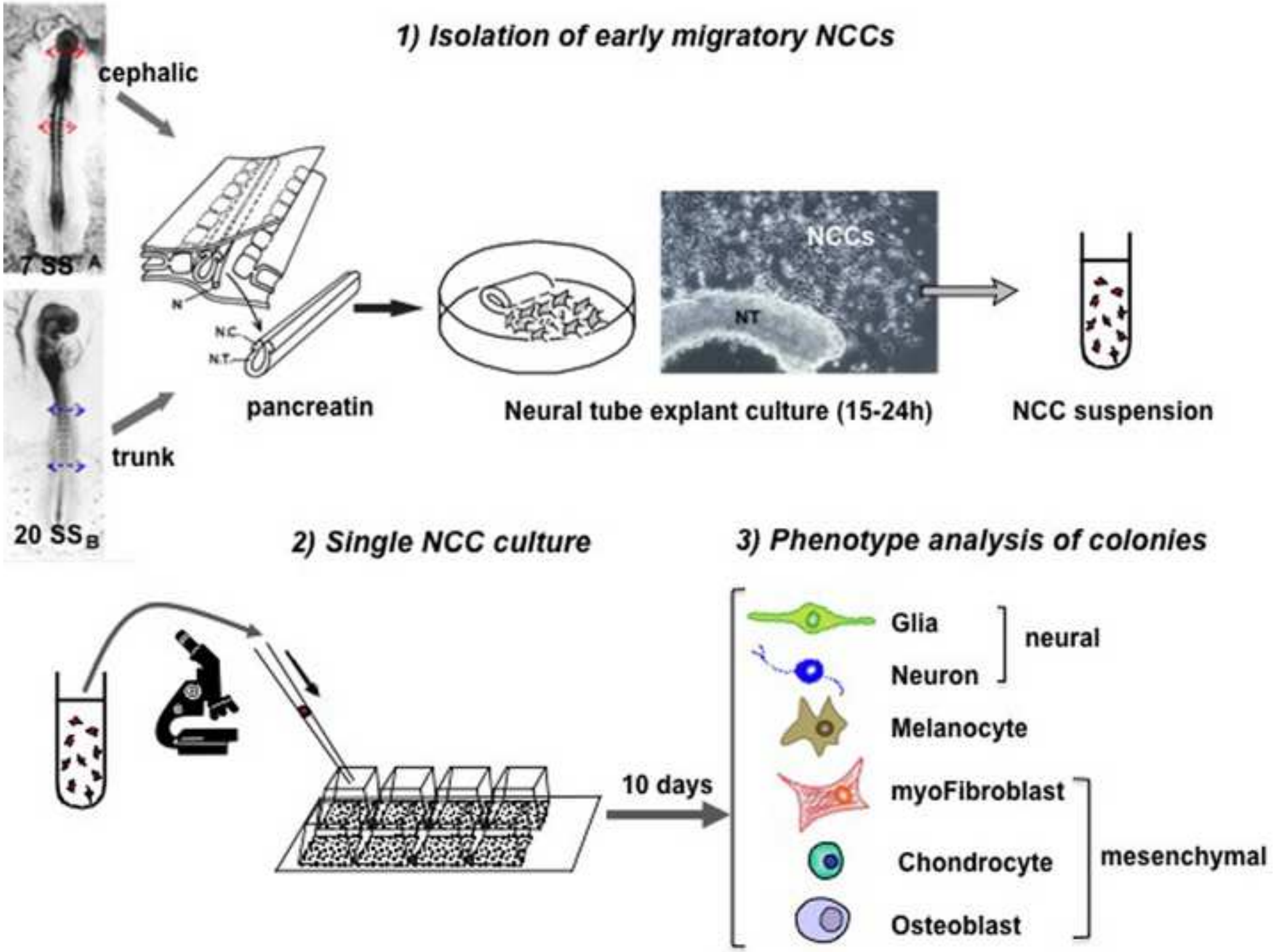

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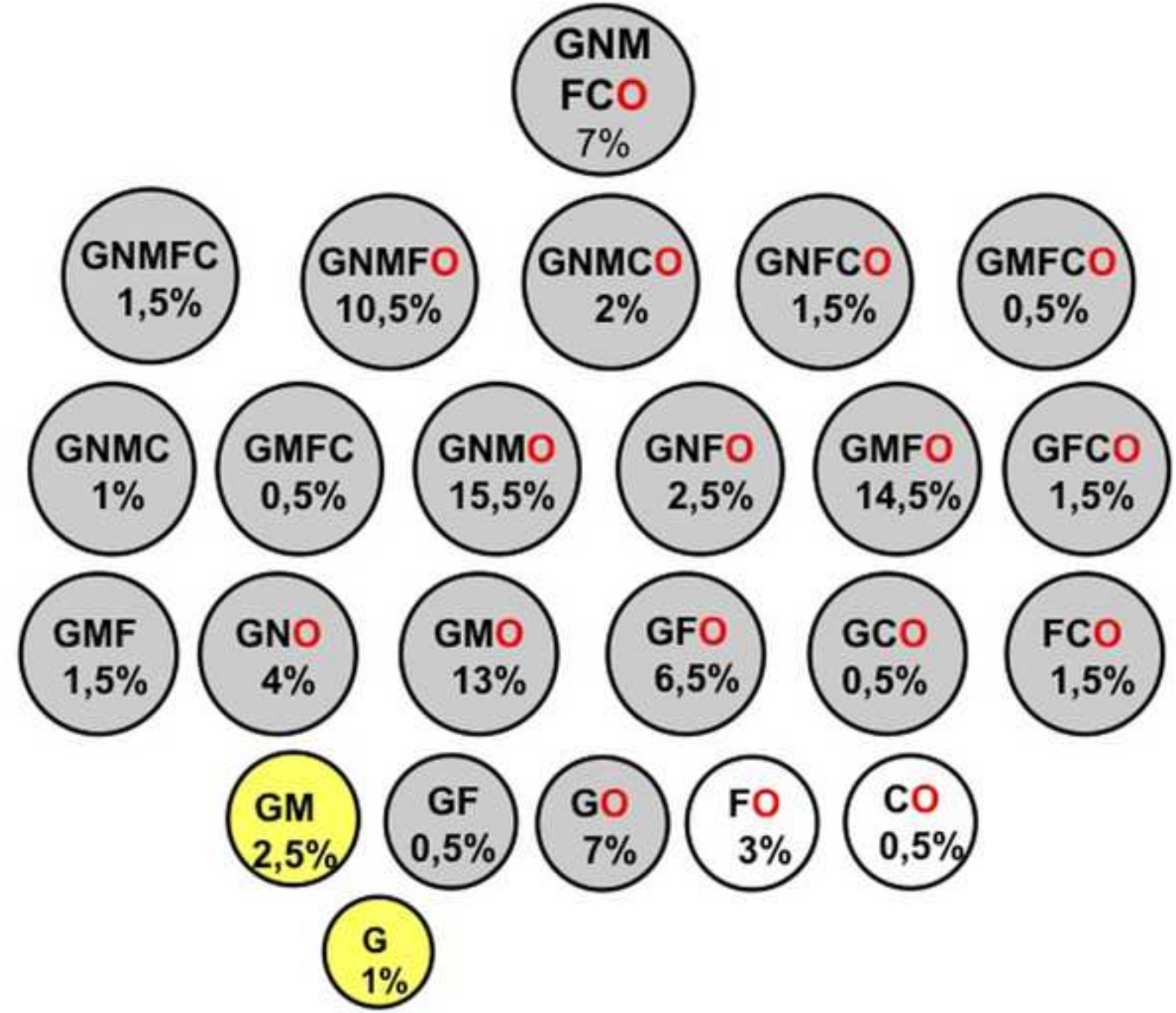

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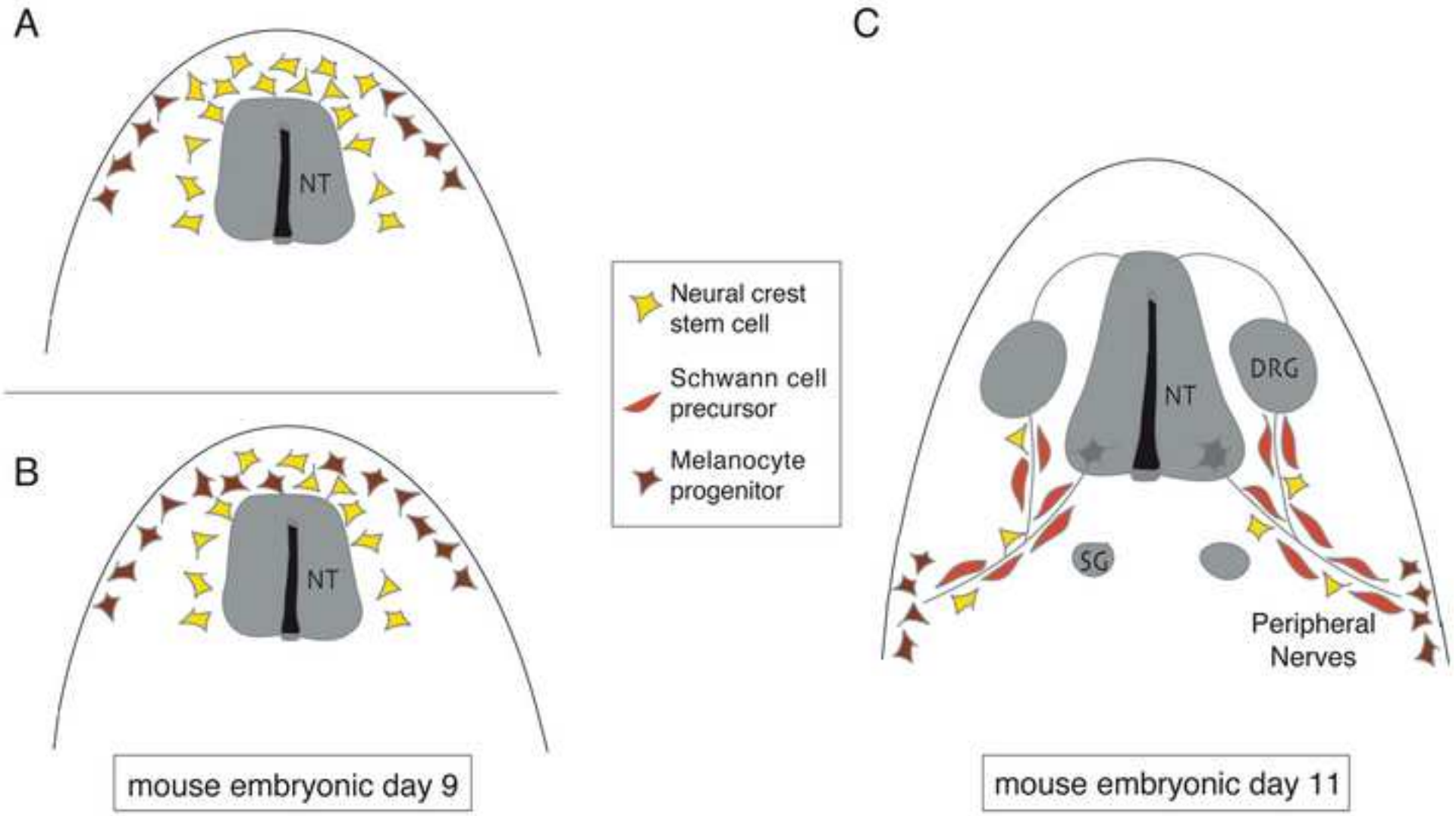

Table

Table 1. Derivatives of the NC in amniote vertebrates

	Cell types			
	Neurons and Glial cells	Pigment cells	Endocrine cells	Mesenchymal cells
TRUNK NC	Sensory ganglia (DRG) Sympathetic ganglia Parasympathetic ganglia Satellite glial cells in ganglia Schwann cells along PNS nerves	Skin melanocytes	Adrenal medullary cells	Endoneurial fibroblasts (mouse sciatic nerve)
CRANIAL NC	Sensory cranial ganglia Parasympathetic (ciliary)ganglia Enteric ganglia Satellite glial cells in ganglia Enteric Glia Schwann cells along PNS nerves Ensheating Olfactory Cells lining the olfactory nerve	Skin melanocytes Pigment cells of the innear ear	Carotid body cells C cells of the ultimobranchial body and thyroid	Cranio-facial skeleton Dermal bone-forming cells Endochondral osteocytes Chondrocytes Other cells in head and neck Myofibroblasts/Smooth muscle cells (conotroncus and aortic arch-derived arteries) Pericytes in brain Meninges (forebrain) Odontoblasts, cellls in periodontial ligament and tooth papillae Adipocytes Dermal cells of the face Connective cells of glands, muscles and tendons Corneal cells in endothelium and stroma Ciliary muscles

Table 2. Postmigratory NC-derived stem cells

Source	*In vivo* Fate Mapping	Expression of Markers	Species and Age	Culture Method	Self-renewal, passage number *in vitro*	Neuronal Potential	Glial Potential	Smooth Muscle Potential	Chondrocyte Osteoblast Adipocyte Potential	References
Sciatic Nerve	no	$p75^+\alpha4^+P_o^-$	rat E14.5-E17.5	adherent, clonal density	2	+	+	+		Morrison et al., 1999
Gut	no	$p75^+\alpha4^+$ $p75^+$(adult)	rat E14.5, adult	adherent, clonal density	2	+	+	+		Bixby et al., 2002 Kruger et al., 2002
DRG	Wnt1-Cre P0-Cre EGFP	$p75^+Sox10^+$	rat E14.5; mouse adult	adherent, clonal density, spheres	2	+	+	+		Hagedorn et al., 1999 Bixby et al., 2002 Nagoshi et al., 2008
Boundary Cap Cells	Krox20-Cre R26R	$p75^+Nestin^+$ Ret^+ $Brn3a^+$ $Krox20^+$	mouse E11.5	spheres	> 6 months	+	+	+		Maro et al., 2004 Hjerling et al., 2005
Bone Marrow	Wnt1-Cre P0-Cre EGFP	$p75^+Sox10^+$ $Slug^+$ $Snail^+$	mouse adult	spheres	2	+	+	+		Nagoshi et al., 2008
Cornea	Wnt1-Cre P0-Cre EGFP	$Sca1^+CD34^+$ $CD45^-$ $C\text{-}kit^-$	mouse adult	spheres	18	+	+	+	+	Yoshida et al., 2006
Heart	P0-Cre EGFP	$Nestin^+Musashi^+$	mouse adult	spheres	n.d.	+	+	+		Tomita et al., 2005
Carotid Body	Wnt1-Cre R26R	$GFAP^+$	mouse adult	spheres	2	+		+		Pardal et al., 2007

Table

Table 3. Multipotent stem cells in the skin identified by mouse genetic fate mapping

Type of Stem Cell	Origin (facial vs. trunk)	Expression of Markers	*In vivo* Fate Mapping	Species and Age	Culture Method	Self-renewal, passage number *in vitro*	Neuronal Potential	Glial Potential	Smooth Muscle Potential	Chondrocyte Osteoblast Adipocyte Potential	Melanocyte Potential	References
NCSCs	facial neural crest	$p75^{+}Sox10^{+}$	Wnt1-Cre R26R	mouse adult	spheres	> 1 year						Wong et al., 2006
SKPs	facial neural crest	$Slug^{+}$ $Snail^{+}$ $Nestin^{+}$ $Sox9^{+}$ $p75^{-}$	Wnt1-Cre R26R Sox2-GFP	mouse juvenile, adult	spheres	> 1 year	+		+			Fernandes et al., 2004 Lavoie et al., 2008 Biernaskie et al., 2009
Epi-NCSCs	facial neural crest	$Nestin^{+}$ $Sox10^{+}$	Wnt1-Cre R26R	mouse adult	hair follicle bulge explants	2	+	+	+	+	+	Sieber-Blum et al., 2004
NCSCs	trunk neural crest	$p75^{+}Sox10^{+}$	Wnt1-Cre R26R Dhh-Cre R26R Dct-Cre R26R	mouse adult	spheres	> 1 year	+	+	+	+	+	Wong et al., 2006
SKPs	*trunk mesoderm*	$Sox2^{+}$	Sox2-GFP; Myf5-Cre R26R, R26YFP	mouse adult	spheres	n.d.	+	+	+	+		Biernaskie et al., 2009 Jinno et al, 2010